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(54) Bacteria-containing product for use in animal feeds, and its production

(57) A product for use as an addition to animal feeds, which contains live bacteria having a useful influence on the intestinal microflora of animals, in which the bacteria are encapsulated in a material of low water content in equilibrium with air of high relative humidity, and in which the material allows the bacteria to be liberated in the digestive tract of animals. The product may be prepared by dispersing the live bacteria in the melted or dissolved encapsulating material at a temperature not exceeding 70°C., and cooling the dispersion by atomisation into an air stream.

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SPECIFICATION

Bacteria-containing product for use in animal feeds, and its production

The present invention relates to a product which is useful in curative or prophylactic treatment of animals by combating pathogenic bacteria in the intestines, or in maintaining normal intestinal microflora, and to a process of producing the product.

It is known that antibiotic treatment of infections in human beings and animals may lead to sterilisation of the intestines, causing intestinal function disorders e.g. diarrhoea. It is also known that an increase of pathogenic bacteria in the intestinal canal usually causes intestinal diseases.

It is of great importance for animal husbandry that the intestinal function of young animals is normal. If it is not, there is a risk of poor weight increase and high mortality.

In the known *Lactobacillus* therapy used to normalise the intestinal function of young animals, non-pathogenic lactic acid-producing bacteria change the intestinal environment in a way which is unfavourable for the pathogenic bacteria, inhibiting their growth and the production of toxins. Curdled milk products have been used for this purpose, but more recently cultured bacterial strains of the family *Lactobacillaceae* have increasingly been used. It also has been reported that *Streptococcus faecium* can compete with haemolytic *coli* bacteria at the low intestinal pH caused by the *Streptococcus* itself.

The curative value of the latter strain is well known, and it would be of great value if this and other strains of similar activity could be made available for prophylactic treatment too by incorporating them in the feed. However, the requirements which a bacteria concentrate for this purpose shall meet are quite strict.

It appears that the stability of a bacteria concentrate, even if stable per se, is seriously reduced when it is incorporated in certain premixes, especially if in equilibrium with air of high humidity, e.g. 65% relative humidity.

In the process of pelletizing feed, the feed is exposed to steam and to compression, resulting in an increase of its temperature, so that in feed preparations containing live bacteria, the number of the bacteria is disastrously reduced during pelletizing of the feed.

It is an object of the present invention to prepare live bacteria concentrates which are stable enough to endure feed pelletizing, and this object is attained by encapsulating live bacteria concentrates with such auxiliary ingredients and in such a way that the coated product can be mixed into a premix at the existing humidity of air without substantially endangering the stability, so that a feed containing such premix may be pelletized, the bacteria killing by the pelleting being kept at a reasonable level.

More specifically, the bacteria are encapsulated in an auxiliary substance, which contains only a small amount of water in equilibrium with air of high relative humidity, the product being formed into particles of convenient size and of a physical strength sufficient to resist external influences during mixing with the feed and during the pelletizing of the product. In this way, most of the bacteria will be permanently enclosed by the auxiliary substance, and only a small number will be in direct contact with the other ingredients in the premix.

Particularly good results in respect of survival during encapsulation and production of premixes are noted when the encapsulated bacteria are of the strains *Streptococcus faecium, Streptococcus faecalis* and *Lactobacillus acidophilus*.

The auxiliary substance to be used for encapsulating the bacteria, being selected from substances which are non-bactericidal and substantially non-toxic, should be solid at room temperature and, preferably, it should melt at least in part at the temperature reached in the feed during the pelletizing process, since the heat consumption in the melting goes towards reducing the temperature, to which the bacteria are exposed in the process. In the choice of auxiliary substances, the heat insulating properties should be taken into account for the same reason.

Preferred auxiliary substances are polyethylene glycols, solid fats, including fatty acid monoglycerides, free fatty acids, fat alcohols, including ethoxylated fat alcohols, and sugars responding to the above criteria.

Particularly preferred auxiliary substances are the solid and semisolid polyethylene glycols marketed under the registered trade mark "Carbowax".

Generally, the auxiliary substances suitable for use in the encapsulating will be to some extent hydrophilic, and care should be taken, that substances are chosen, containing only a small amount of water, since a high water content reduces the stability of the bacteria preparation.

To illustrate this effect of the water content, four products with different water contents were made, the water content of each one in equilibrium with 65% r.H. The stability of the four products was compared with that of the untreated bacteria concentrate. All experiments were carried out at 65% r.H. and in a vitamin mineral premix, which has been found to be aggressive against the bacteria. The results are given in table 1, and the composition of the vitamin mineral

premix is given in table 2.

Table 1

	equilibrium water content at 65% r.H.	average decrease per week 9–12 w.
Product 1	9.7	62%
Product 2	3.8	24%
Product 3	3.2	18%
Product 4	0.9	5%
Untreated		54%

Table 2

Vitamin A 500,000 IU/g	1.54 g
Vitamin A 500,000 IU/g + vitamin D_3 167,000 IU/g	0.67 g
Vitamin E-adsorb. 50%	12.00 g
Vitamin B_{12} of 1000 mg/kg	4.00 g
Niacin	3.00 g
Calcium pantothenate	3.00 g
Pyridoxine HCl	0.80 g
Riboflavine	0.80 g
Thiamine mononitrate	0.40 g
Lysine	48.00 g
Methionine	32.00 g
Cobalt sulfate	0.40 g
Zinc carbonate	14.60 g
Manganese oxide	12.40 g
Copper sulfate	40.00 g
Ferrous sulfate	25.00 g
Calcium magnesium carbonate	400.00 g
Feeding wheat flour	399.00 g
Lactiferm (concentrate of *Strept. faecium*)	2.00 g
	1000.00 g

The figures of Table 1 indicate that the decrease in the number of live bacteria in the preparation is inversely proportional to the water content of the auxiliary substances, and that the water content should preferably not exceed 4% and most preferably be below 1%.

As stated above, the auxiliary ingredient must be a non-bactericidal and non-toxic substance with a low equilibrium water content at 65% r.H. In addition a small amount of inert additives, such as binders, may be useful. If the auxiliary ingredient does not solidify immediately, a powdering with talc or other auxiliaries may be applied.

In Table 3 below is given the percentage decrease per month in the number of live bacteria, which has been observed when using various encapsulating substances, binders and powders if the products are stored in a normal atmosphere.

Table 3

Encapsulation material	Binder	Powder	Decrease in number of live bacteria per month
Sugar	Hydroxyethyl cellulose	Talc	5%
Sugar	Hydroxyethyl cellulose	Corn starch hydrophilic	6%
Sugar	Agar	Lactose	9%
Sugar	Gelatin	Talc	6%
Stearic acid monoglyceride			5%
Palmitic acid			4%
Hydrogenated palm-kernel oil			5%
Polyethylene glycol 6000			1%
Not encapsulated			24%

Tests have further been carried out to determine the stability of products containing different bacteria encapsulated in the same auxiliary ingredient together with a mixture of vitamins and minerals as specified in Table 2, and stored in air of 65% relative humidity. The encapsulating material was sugar with hydroxyethylcellulose as binder and powdered with talc. The results of the tests appear from Table 4 below.

Table 4

Bacteria	Decrease of live bacteria per month
Streptococcus faecium	19%
Lactobacillus acidophilus	34%
Streptococcus faecalis	39%
Streptococcus faecium in mixture with vitamins and minerals, but not encapsulated	70%

The present bacterial products can be directly mixed with animal feeds. In practice, however, it is generally preferred that the live bacteria encapsulated together with vitamins and minerals are incorporated in a premix with part of the feed, and preferably the premix is in pelletized form. The pellets can then be mixed with the remainder of the feed, thus ensuring the substantially even distribution of the live bacteria in the feed which is necessary for a controlled dosing of the individual animal.

The production of the present bacterial products is generally carried out in the following manner. A concentrate of live bacteria is dispersed in a melted or dissolved encapsulating material at a temperature not exceeding 70°C, said material being of low water content in equilibrium with air of high relative humidity, after which the dispersion is cooled by being atomized into an air stream.

When using a water-soluble encapsulating material, e.g. sugar, together with a soluble binder, it may be expedient to powder the particles containing the live bacteria with a hydrophobic substance, e.g. talc, which to some extent protects against the influence of steam during pelletizing. For example, the talc can be contained in a cold air stream used for cooling the atomized dispersion.

Using encapsulating materials which are solid at room temperature but melting below 70°C. ensures on one hand that the bacteria are not substantially damaged by being dispersed in the melted material, and on the other hand that heat is consumed by melting of the material, if such melting occurs by pelletizing, the encapsulating material thus acting as a buffer against increase

of temperature during pelletizing.

Appropriate embodiments of the present process are illustrated by the following Examples.

Example 1

2 kg of mixture specified in Table 2, containing 380 × 10^9 bacteria per g are stirred into 3 l of water at room temperature.

In another vessel, 12 kg of sugar are dissolved in 7 l of water at 60°C, and a previously prepared solution of 17 g of agar in 0.5 l of boiling water is added. Then, the slurry of the bacteria-containing mixture is mixed into the solution of sugar and agar, the temperature of the resulting mixture being 52–53°C.

Using a centrifugal atomizer, the mixture is then atomized at a rate of 0.7 l/minute into a spray chamber countercurrently to air of room temperature containing a powdering agent, e.g. hydrophic corn starch. The spray chamber is combined with a fluid bed-unit, in which the product is dried and excess of powdering agent blown away.

The yield is 14.35 kg with a content of 40 × 10^9 bacteria per g. The content of powdering agent is approximately 14%.

In this manner, the products of Table 3 were produced, having sugar as the encapsulating material.

Example 2

150 g of Carbowax 20M are melted and kept at 60°C while into the melted mass are stirred 1.5 g of a concentrate of *Streptococcus faecium* containing 360 × 10^9 bacteria per g. The melt is then solidified by pouring it in a thin layer onto a cold plate, and the solidified product is comminuted and screened through a screen with a mesh width of 0.42 mm (US Mesh 40).

22.7 g of the resulting product, containing 2.2 × 10^9 bacteria per g, are mixed into 100 kg of feed and pelletized industrially.

In this manner, the products of Table 5 below encapsulated in Carbowax with and without talc have been produced. The table shows how the encapsulation improves the stability of the bacteria concentrate during pelletizing.

Table 5

Bacteria concentrate treated with	Number of live bacteria per g	
	Before pelletizing	After pelletizing
Talc alone	500,000	29,000
Carbowax 20M alone	500,000	166,000
Talc and Carbowax	500,000	120,000
Untreated	500,000	13,000

Example 3

In the manner described in Example 1 there is produced from

40 g of the mixture of Table 2
608 g of sugar
1.6 g of hydroxyethylcellulose, and
375 g of water,

a dispersion which is atomized into a cold air stream containing talc.

450 g of the resulting product are moistened with a solution of 450 g of Carbowax 4000 in 450 g of acetone, mixed with 350 g of talc, and dried.

The resulting product contains 0.97 × 10^9 bacteria per g.

619 g of the product ar mixed into 20 kg of wheat feed meal. This premix is mixed into 3000 kg of a feed mixture, and feed pellets are produced therefrom.

The viability of the bacteria in these pellets as compared with a corresponding product, in which the bacteria have not been encapsulated, will appear from Table 6 below.

Table 6

	Bacteria encapsulated in Carbowax and talc	Non-encapsulated bacteria
Number of live bacteria per g	200.000	200.000
Just after pelletizing	62.000	3.900
After 5 weeks	36.000	1.400
After 10 weeks	40.000	1.300
After 15 weeks	26.000	1.500

Further, tests were carried out, using different forms of encapsulating, the percentage loss of live bacteria by pelletizing being determined.

The tests were made with the mixture of Table 2, and the various products were encapsulated as described in Example 1. The resulting products were mixed with feed and stirred into boiling water in the proportions 1 g of the bacteria product to 100 g of feed and 25 g of boiling water, and the warm mixture (36°C) was pelletized, during which the temperature increased to 58.5°C.

Table 7 below specifies encapsulating materials and loss of bacteria by the pelletizing.

Table 7

Encapsulation material	Binder	Powder	Loss of live bacteria by pelletizing
Cetyl alcohol		talc	19%
Sugar with 20% of polyethylene glycol			0%
Sugar	Agar	polyethylene glycol and talc	26%
Ethoxylated fatty alcohol			37%
Not encapsulated			62%

CLAIMS

1. A product containing live bacteria which can usefully influence the intestinal microflora of an animal, in which the bacteria are encapsulated in a material of low water content in equilibrium with air of high relative humidity, and in which the material allows the bacteria to be liberated in the digestive tract of the animal.
2. A product according to claim 1 in which the encapsulated bacteria are selected from the species *Streptococcus faecium, Streptococcus faecalis* and *Lactobacillus acidophilus*.
3. A product according to claim 1 or claim 2 in which the encapsulating material is selected from polyethylene glycols, solid fats, free fatty acids, fatty alcohols and sugars.
4. A product according to any preceding claim in which the water content of the encapsulating material is less than 10% in air of 65% relative humidity.
5. A product according to claim 4 in which the water content is less than 8%.
6. A product according to claim 4 in which the water content is less than 6%.
7. A product according to claim 4 in which the water content is not more than 4%.
8. A product according to claim 4 in which the water content is less than 1%.
9. A product according to any preceding claim in which the bacteria are encapsulated together with vitamins, minerals and/or feed components for the animal.
10. A product according to any preceding claim in the form of pellets.
11. A product according to claim 1 substantially as described in any of the Examples.
12. A method for preparing a product according to any preceding claim which comprises dispersing a concentrate of the live bacteria in melted or dissolved encapsulating material at a temperature not exceeding 70°C., and cooling the dispersion by atomisation into an air stream.
13. An animal feed composition comprising a product according to any of claims 1 to 11.

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